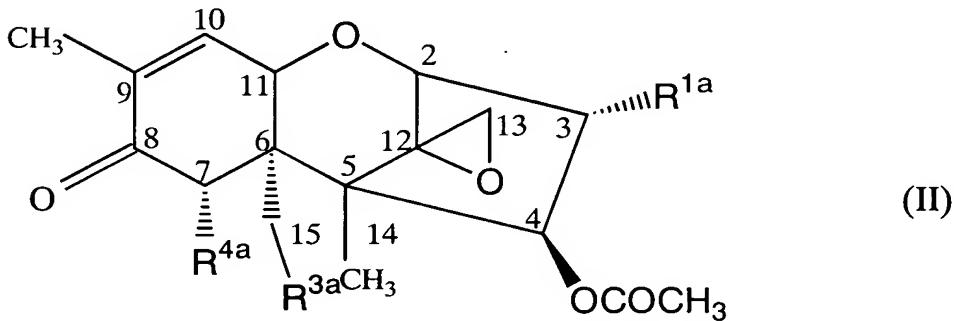


## CLAIMS

1. A monoclonal antibody or a fragment thereof which has affinity for compounds represented by formula (II):

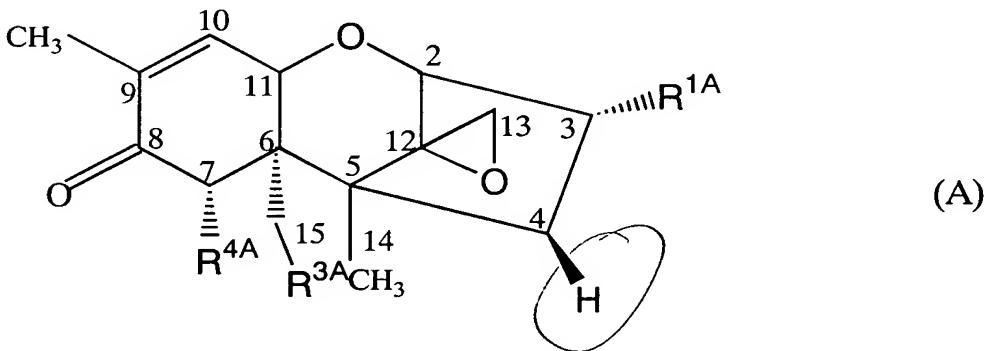
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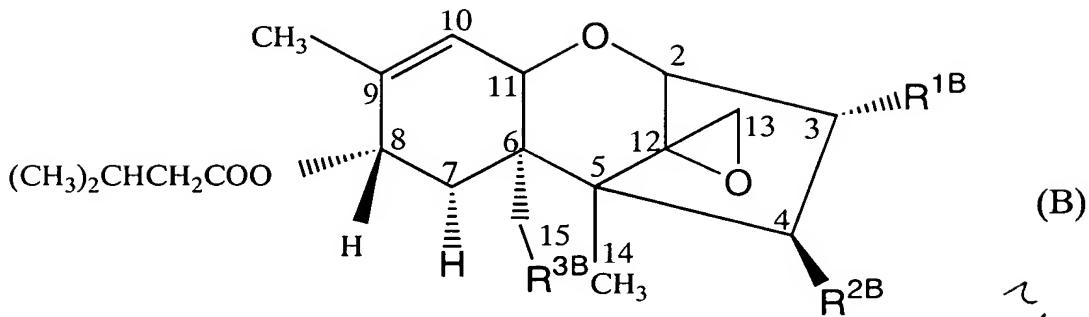
Must be prepared using immunogen (I)  
/reproducible?

(wherein  $R^{1a}$ ,  $R^{3a}$  and  $R^{4a}$ , which may be the same or different, each represents  $\text{OH}$  or  $\text{acyloxy}$ ) and which does not substantially react with compounds represented by formula (A):

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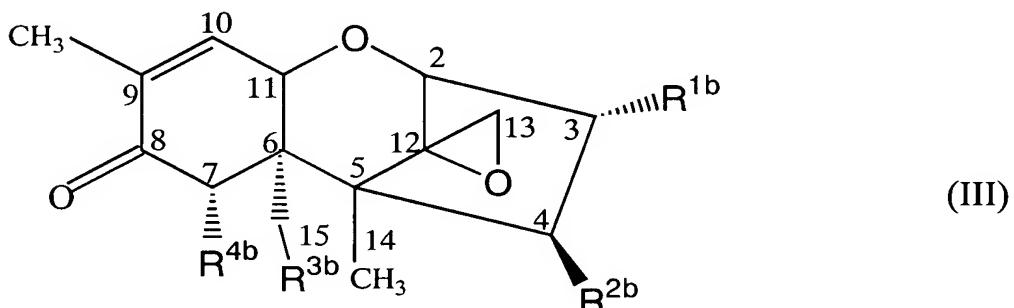
15 (wherein  $R^{1A}$ ,  $R^{3A}$  and  $R^{4A}$ , which may be the same or  
different, each represents OH or acyloxy) or compounds  
represented by formula (B):



(wherein  $R^{1B}$ ,  $R^{2B}$  and  $R^{3B}$ , which may be the same or different, each represents OH or acyloxy), said affinity for the compounds represented by formula (II) decreasing in the order of Compound 1-1 > Compound 1-2 > Compound 1-3, wherein Compound 1-1 is a compound represented by formula (II) in which  $R^{1a}$  and  $R^{3a}$  are  $OCOCH_3$  and  $R^{4a}$  is OH, Compound 1-2 is a compound represented by formula (II) in which  $R^{1a}$  and  $R^{4a}$  are OH and  $R^{3a}$  is  $OCOCH_3$ , and Compound 1-3 is a compound represented by formula (II) in which  $R^{1a}$ ,  $R^{3a}$  and  $R^{4a}$  are  $OCOCH_3$ .

2. The monoclonal antibody or a fragment thereof according to claim 1, wherein the monoclonal antibody is monoclonal antibody KTM-205 produced by hybridoma KTM-205.

3. A monoclonal antibody or a fragment thereof which has affinity for compounds represented by formula (III):

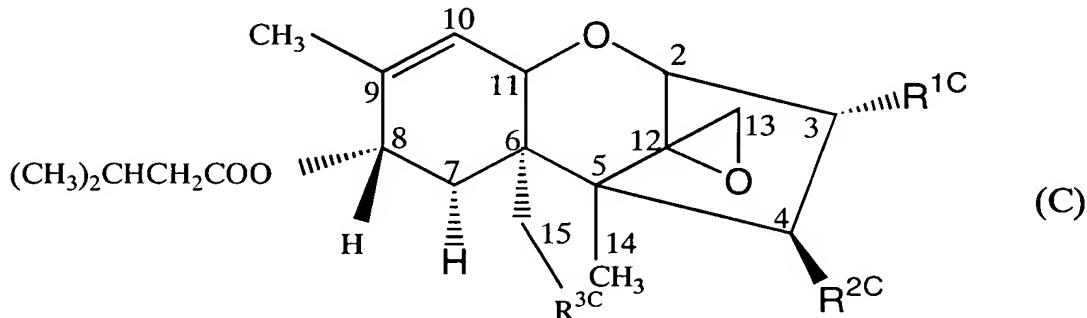


(wherein  $R^{1b}$ ,  $R^{2b}$  and  $R^{4b}$ , which may be the same or different, each represents OH or acyloxy; and  $R^{3b}$

KTM-205 antibody

represents H, OH or acyloxy, provided that when R<sup>2b</sup> is H or OH, at least one of R<sup>1b</sup>, R<sup>3b</sup> and R<sup>4b</sup> is acyloxy) and which does not substantially react with compounds represented by formula (C):

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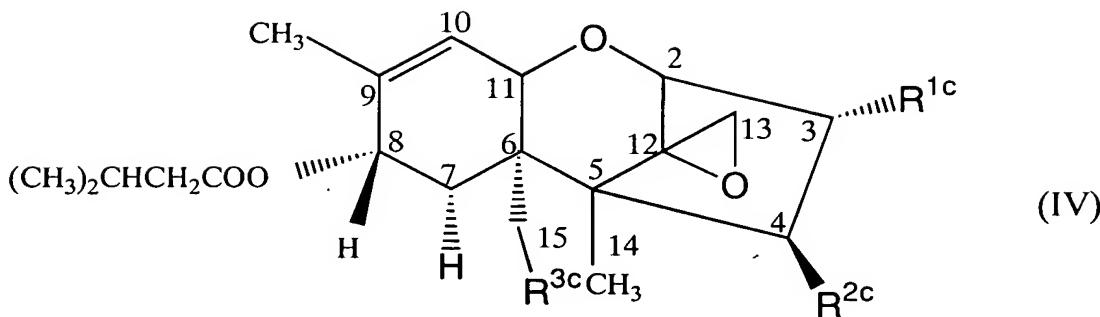
(wherein R<sup>1C</sup>, R<sup>2C</sup> and R<sup>3C</sup>, which may be the same or different, each represents OH or acyloxy), said affinity 10 for the compounds represented by formula (III) decreasing in the order of Compound 2-1 > Compound 2-2 > Compound 2-3, wherein Compound 2-1 is a compound represented by formula (III) in which R<sup>1b</sup> and R<sup>3b</sup> are OCOCH<sub>3</sub>, R<sup>2b</sup> is H and R<sup>4b</sup> is OH, Compound 2-2 is a compound represented by formula 15 (III) in which R<sup>1b</sup>, R<sup>3b</sup> and R<sup>4b</sup> are OCOCH<sub>3</sub> and R<sup>2b</sup> is H, and Compound 2-3 is a compound represented by formula (III) in which R<sup>1b</sup> and R<sup>4b</sup> are OH and R<sup>2b</sup> and R<sup>3b</sup> are OCOCH<sub>3</sub>.

4. The monoclonal antibody or a fragment thereof 20 according to claim 3, wherein the monoclonal antibody is monoclonal antibody KTM-240 produced by hybridoma KTM-240.

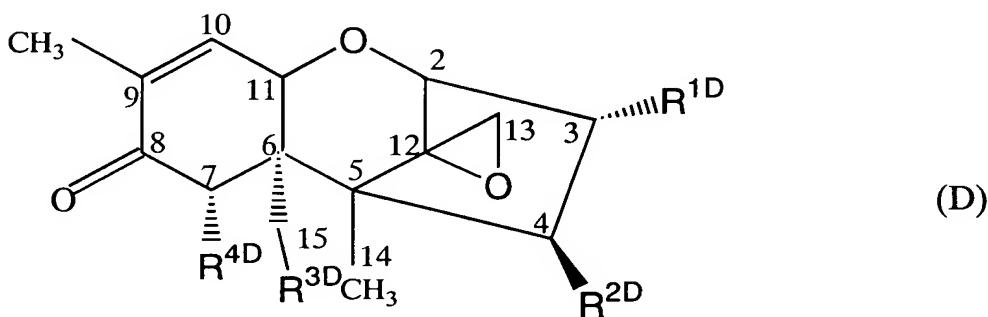
5. A monoclonal antibody or a fragment thereof which has affinity for compounds represented by formula (IV):

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(wherein  $R^{1c}$ ,  $R^{2c}$  and  $R^{3c}$ , which may be the same or different, each represents OH or acyloxy, provided that at least one of  $R^{1c}$ ,  $R^{2c}$  and  $R^{3c}$  is acyloxy) and which does not substantially react with compounds represented by formula (D):



(wherein  $R^{1D}$ ,  $R^{3D}$  and  $R^{4D}$ , which may be the same or different, each represents OH or acyloxy, and  $R^{2D}$  is H, OH or acyloxy), said affinity for the compounds represented by formula (IV) decreasing in the order of Compound 3-1 > Compound 3-2, wherein Compound 3-1 is a compound represented by formula (IV) in which  $R^{1c}$  is OH and  $R^{2c}$  and  $R^{3c}$  are  $OCOCH_3$ , and Compound 3-2 is a compound represented by formula (IV) in which  $R^{1c}$ ,  $R^{2c}$  and  $R^{3c}$  are  $OCOCH_3$ .

6. The monoclonal antibody or a fragment thereof according to claim 5, wherein the monoclonal antibody is monoclonal antibody KTM-249 produced by hybridoma KTM-249.

7. A hybridoma which is capable of producing the

monoclonal antibody according to claim 1 or 2.

8. A hybridoma which is capable of producing the monoclonal antibody according to claim 3 or 4.

5

9. A hybridoma which is capable of producing the monoclonal antibody according to claim 5 or 6.

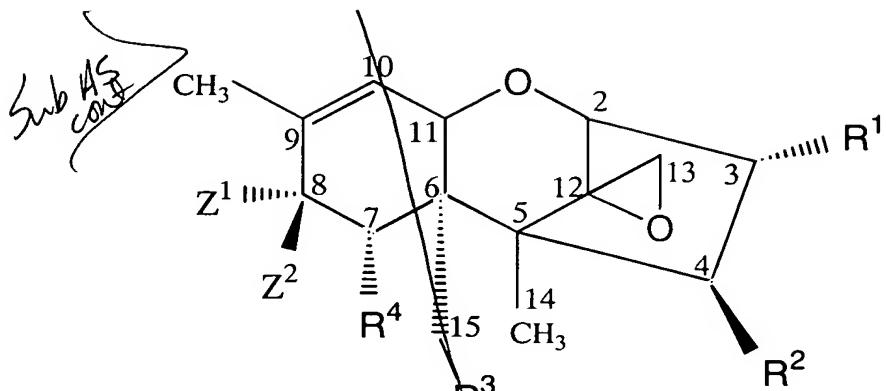
10. The hybridoma according to claim 7 deposited with  
the National Institute of Bioscience and Human-Technology,  
Agency of Industrial Science and Technology under  
accession number FERM BP-6835.

11. The hybridoma according to claim 8 deposited with  
the National Institute of Bioscience and Human-Technology,  
Agency of Industrial Science and Technology under  
accession number FERM BP-6836.

12. The hybridoma according to claim 9 deposited with  
the National Institute of Bioscience and Human-Technology,  
Agency of Industrial Science and Technology under  
accession number FERM BP-6837.

13. A process for producing a hybridoma which  
produces the monoclonal antibody according to any of  
claims 1 to 6, which comprises immunizing an animal by  
administering to the animal a substance prepared from a  
compound represented by formula (I):

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(I)

(wherein R<sup>1</sup> represents H or acyloxy; R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup>, which may be the same or different, each represents H, OH or acyloxy; and Z<sup>1</sup> represents OCOCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub> and Z<sup>2</sup> represents H, or Z<sup>1</sup> and Z<sup>2</sup> together represent =O, provided that at least one of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> is OH) by converting at least one of the hydroxyl groups therein to acyloxy and binding a carrier substance to the carbon at the 3-position thereof, and fusing an antibody-producing cell obtained from the immunized animal with a permanent growth cell to obtain the hybridoma.

14. The process according to claim 13, wherein R<sup>2</sup> in formula (I) is acyloxy.

15. The process according to claim 13, wherein the binding of the carrier substance to the carbon at the 3-position of a compound prepared from the compound represented by formula (I) by converting at least one of the hydroxyl groups therein to acyloxy is carried out by using a substituent at the 3-position as a linker.

16. The process according to claim 13, wherein a compound prepared from the compound represented by formula (I) by converting at least one of the hydroxyl groups therein to a group represented by OR (wherein R represents a substituted or unsubstituted lower acyl group or a

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substituted or unsubstituted aromatic acyl group) is dissolved in a solvent which is not an organic solvent or which does not contain an organic solvent and then conjugated to the carrier substance.

5

17. The process according to claim 16, wherein the solvent which is not an organic solvent or which does not contain an organic solvent is water.

10

*Method steps*  
18. An immunoassay for determining a trichothecene mycotoxin in a sample, which comprises causing at least one of the monoclonal antibodies and fragments thereof according to claims 1 to 6 to act on the sample containing the trichothecene mycotoxin.

15

19. An immunoassay for determining a trichothecene mycotoxin in a sample, which comprises converting at least one hydroxyl group in a compound represented by formula (I) having at least one hydroxyl group to a group represented by OR (wherein R represents a substituted or unsubstituted lower acyl group or a substituted or unsubstituted aromatic acyl group), and causing at least one of the monoclonal antibodies and fragments thereof according to claims 1 to 6 to act on the obtained compound.

25

*Sub A/B*  
20. The immunoassay according to claim 18 or 19, wherein the trichothecene mycotoxin is selected from the group consisting of deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2) and derivatives thereof.

30

21. A method for determining the total amount of DON, NIV, T-2 and derivatives thereof in a sample, which comprises calculating the total amount from the value obtained by the immunoassay according to claim 18 or 19 using the monoclonal antibody or a fragment thereof according to claim 3 or 4 and the value obtained by the

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*Sub A v. cont* immunoassay according to claim 18 or 19 using the monoclonal antibody or a fragment thereof according to claim 5 or 6.

5 22. A method for determining NIV and its derivatives in a sample, which comprises carrying out the immunoassay according to claim 18 or 19 using the monoclonal antibody or a fragment thereof according to claim 1 or 2.

10 23. A method for determining DON, NIV and derivatives thereof in a sample, which comprises carrying out the immunoassay according to claim 18 or 19 using the monoclonal antibody or a fragment thereof according to claim 3 or 4.

15 24. A method for determining DON and its derivatives in a sample, which comprises calculating the amount of DON and its derivatives from the value obtained by the immunoassay according to claim 18 or 19 using the 7. monoclonal antibody or a fragment thereof according to claim 3 or 4 and the value obtained by the immunoassay according to claim 18 or 19 using the monoclonal antibody or a fragment thereof according to claim 1 or 2.

25 25. A method for determining T-2 and its derivatives in a sample, which comprises carrying out the immunoassay according to claim 18 or 19 using the monoclonal antibody or a fragment thereof according to claim 5 or 6.

30 26. The immunoassay according to claim 18 or 19, wherein the immunoassay is selected from the group consisting of radioimmunoassay, enzyme immunoassay, fluoroimmunoassay and luminescence immunoassay.

35 27. The immunoassay according to claim 18 or 19, wherein the immunoassay is selected from the group

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consisting of competitive immunoassay and sandwich immunoassay.

28. A reagent for immunoassay for determining a  
5 trichothecene mycotoxin, comprising at least one of the  
monoclonal antibodies and fragments thereof according to  
claims 1 to 6 as an active ingredient.

29. A kit for immunoassay for determining a  
10 trichothecene mycotoxin, comprising the reagent according  
to claim 28 and an antigen-immobilized solid phase plate.

*Sub A<sup>1</sup>* 30. A kit for immunoassay for determining a  
trichothecene mycotoxin, comprising the reagent according  
15 to claim 28, an antigen-immobilized solid phase plate, a  
labeled antibody or antibody fragment which reacts with  
the monoclonal antibody or a fragment thereof according to  
any of claims 1 to 6, and a reagent for detecting the  
label of said antibody or antibody fragment.

20  
31. A kit for immunoassay for determining a  
trichothecene mycotoxin, comprising an antigen-immobilized  
solid phase plate, the monoclonal antibody or a fragment  
thereof according to any of claims 1 to 6 which is labeled,  
25 and a reagent for detecting the label of said antibody or  
antibody fragment.

32. A kit for immunoassay for determining a  
trichothecene mycotoxin, comprising the reagent according  
30 to claim 28 and a solution for the pretreatment of a  
sample to convert a hydroxyl group in a compound  
represented by formula (I) to a group represented by OR  
(wherein R has the same significance as defined above).

35  
33. A kit for immunoassay for determining a  
trichothecene mycotoxin, comprising the reagent according

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to claim 28, an antigen-immobilized solid phase plate, and a solution for the pretreatment of a sample to convert a hydroxyl group in a compound represented by formula (I) to a group represented by OR (wherein R has the same significance as defined above).

*Sub A8* 34. A kit for immunoassay for determining a trichothecene mycotoxin, comprising the reagent according to claim 28, an antigen-immobilized solid phase plate, a labeled antibody or antibody fragment which reacts with the monoclonal antibody or a fragment thereof according to any of claims 1 to 6, a reagent for detecting the label of said antibody or antibody fragment, and a solution for the pretreatment of a sample to convert a hydroxyl group in a compound represented by formula (I) to a group represented by OR (wherein R has the same significance as defined above).

35. A kit for immunoassay for determining a trichothecene mycotoxin, comprising an antigen-immobilized solid phase plate, the monoclonal antibody or a fragment thereof according to any of claims 1 to 6 which is labeled, a reagent for detecting the label of said antibody or antibody fragment, and a solution for the pretreatment of a sample to convert a hydroxyl group in a compound represented by formula (I) to a group represented by OR (wherein R has the same significance as defined above).

*Sub A9* 36. A method for determining a trichothecene mycotoxin in a sample, which comprises treating the sample containing the trichothecene mycotoxin with a solution containing an organic solvent to extract the trichothecene mycotoxin from the sample, and determining the extracted trichothecene mycotoxin by the immunoassay according to claim 18 or 19.

37. The method according to claim 36, wherein the organic solvent is a water-soluble organic solvent.

~~Sub A~~ 38. The method according to claim 36 or 37, wherein the water-soluble organic solvent is at least one member selected from the group consisting of methyl alcohol, ethyl alcohol, propyl alcohol, acetonitrile, dimethyl sulfoxide and dimethylformamide.

10 39. A method for detecting a microorganism producing a trichothecene mycotoxin in a sample by immunoassay, which comprises inoculating the sample containing the microorganism producing the trichothecene mycotoxin into a medium, culturing the microorganism in the medium, and  
15 causing at least one of the monoclonal antibodies and fragments thereof according to claims 1 to 6 to act on the trichothecene mycotoxin produced in the culture.

20 40. A method for identifying a microorganism producing a trichothecene mycotoxin in a sample by immunoassay, which comprises inoculating the sample containing the microorganism producing the trichothecene mycotoxin into a medium, culturing the microorganism in the medium, and causing at least one of the monoclonal  
25 antibodies and fragments thereof according to claims 1 to 6 to act on the trichothecene mycotoxin produced in the culture.

*Add AII*